

to specific RNA motifs. In the cell, however, the stability of the folded RNA depends on its interactions with many other solutes. Using small angle X-ray scattering to measure the folding of a bacterial group I ribozyme, we showed that molecular crowding equivalent to what is present in real cells stabilizes RNA tertiary structures by several kcal/mol. We used polyethylene glycol (PEG) with different molecular weights as a crowding agent, as it does not appreciably interact with the RNA. Stabilization of the native RNA in MgCl₂ or NaCl is most likely due to the excluded volume effect, and as our results were not explained by changes to water or ion activity. Crowder molecules favor more compact structures of the unfolded and native RNAs. Furthermore, compression of the native state ensemble correlates with an increase in ribozyme catalytic activity. Consequently, the ribozyme reaches its catalytically active structure at much lower Mg²⁺ concentrations in a crowded milieu than in a dilute solution. Thus, our results partly explain why many ribozymes are more active in the crowded environment of the cell than in the test tube.

18-Subg

Understanding Protein & RNA Biophysics in Cells

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I will discuss the successes and challenges of understanding the properties of proteins and RNA in cells and under crowded conditions in vitro. For proteins, weak nonspecific interactions can overcome the stabilizing excluded volume effect. For RNA, our recent work using in-cell SHAPE (Selective 2'-Hydroxyl Acylation analyzed by Primer Extension) chemistry shows that the intracellular environment has a large effect on flexibility and structure of the adenine riboswitch.

Subgroup: Nanoscale Biophysics

19-Subg

“Nanoscopium Nominare Libuit”: Approaches Towards Optical Nanoscopy and Individual Molecule Localization Microscopy Improvements

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A recognized advantage of optical microscopy, since Galileo Galilei's “occhialino” times, lies in the fact that allows non-invasive three-dimensional (3D) imaging of live cells at the submicron scale with high specificity and localization accuracy. In general, is a well-known paradigm the given inability of a lens-based optical microscope to discern details that are closer together than half of the wavelength of light. Recently, the viewpoint for improving resolution moved from optical solutions to the side of the fluorescent molecule to be detected taking into account the concepts raised by Toraldo di Francia in the 50s and Lukosz late in the 60s. Today, for the most popular imaging mode in optical microscopy, i.e. fluorescence, the diffraction barrier is crumbling and the term “optical nanoscopy”, coined earlier, comes to be a real far field optical microscope available for the scientific community as the ones allowing individual molecule localization at high precision. We will discuss targeted and stochastic readout methods using both single and multiphoton excitation, in terms of resolution and localization precision accuracy. As well, individual molecule 3D super resolution within selective plane illumination and multiphoton excitation STED will be addressed towards applications in thick biological samples. Furthermore, optical nanoscopy integration with scanning probe methods will be also addressed. A variety of architectures will be outlined in regard to specific applications demanding for nanoscale investigations. Work supported by IIT, IFOM and Italian PRIN 2008JZ4MLB.

20-Subg

Nanocrystal Molecules with Applications in Single Molecule Biological Imaging

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Over the previous decade, new techniques emerged which permit the synthesis of inorganic nanocrystals with well controlled site and shape and even connectivity (branched) and topology (nested). These nanocrystals exhibit strangely size dependent properties, and can be considered a type of “artificial atom,” with controlled density of states. It is by now well established that these nanoparticles can be used in a wide variety of biological imaging applications. Our current work focuses on the creation of “nanocrystal molecules” in which

specific groups of nanocrystals are brought together, producing a collective response. These nanocrystal molecules can, in turn, be used to sense biological events at the molecular lever and can form the basis of new types of local force sensors.

21-Subg

Probing Cell Nanoscale Structural Properties Using Intrinsic Contrast of Light Scattering

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There has been a pressing need to quantify the cellular and sub-cellular structures at the nanoscale in its natural environment. Light scattering represents the pre-dominant intrinsic light interaction with cells. The fundamental diffraction barrier often prevents the access to the structural components approximately less than the scale of the wavelength. The complex structure of an object can be described by 3D refractive index distribution (i.e., scattering potential) or its Fourier spectrum (spatial frequency). Based on the analysis of the spectral-spatial properties of scattered light during light-structure interaction, we introduce a new physical approach to probe the complex structural characteristics at the nanoscale with scattered light. Our approach encodes a specific spatial frequency with a corresponding wavelength, which produces a super-resolved microscopic image and allows structural quantification of a sub-cellular component with nanoscale sensitivity and accuracy. We first validated the feasibility of this approach to probe nanoscale structural properties using model systems. Then we investigated the nano-structural properties from cell nucleus during the cell cycle. The translational potential of this approach was also demonstrated for early cancer detection using clinical cell specimens from patients with pre-cancerous lesions.

22-Subg

Single-Molecule Studies of Trapped Biomolecules in Solution with the ABEL Trap

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Studies of single fluorescent molecules, proteins, or enzymes has provided a wealth of physical and chemical information on the nanoscale devoid of ensemble averaging. To study a single biomolecule in solution without surface attachment or confinement, the Anti-Brownian ELectrokinetic (ABEL) trap provides real-time suppression of Brownian motion, and provides time-averaged uniform pumping intensity for long-time observation without the use of laser tweezers (Cohen and Moerner, Opt. Express (2008)). In recent applications, we have explored the photodynamics of the antenna protein allophycocyanin (Goldsmith et al., Nature Chem. (2010)) and extracted ATP number distributions for single multi-subunit chaperonin enzymes (Jiang et al., PNAS (2011)). This device has also been used to follow electron transfer dynamics for individual nitrite reductase enzymes (Goldsmith et al., PNAS (2011)), providing a new window into light-driven photodynamics, cooperativity, and enzymatic mechanisms.

23-Subg

Diamond Sensors for Intracellular Processes, Protein Motion and Biomagnetic Fields

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Due to their low cytotoxicity, high photostability and simultaneously small size color center doped diamond nanocrystals promise to be versatile bio labels [1]. In additions they are robust donors for FRET processes and allow for detection of pH changes [2]. Since some color centers are paramagnetic they are even efficient sensors for small electric and magnetic field. This for example might allow for the detection of weak currents caused by the ion flow through individual channels [3]. The talk will summarize recent advances and provide examples for biosensing application of diamond defects [4].

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